Trimodality Treatment in Stage III Nonsmall Cell Lung Carcinoma

*Prognostic Impact of K-*ras *Mutations after Neoadjuvant Therapy*

Petra Broermann, M.D.[1]
Klaus Junker, M.D.[2]
Burkhard H. Brandt, Ph.D.[3]
Achim Heinecke, Ph.D.[4]
Lutz Freitag, M.D.[5]
Folker Klinke, M.D.[6]
Wolfgang E. Berdel, M.D.[1]
Michael Thomas, M.D.[1]

[1] Department of Medicine/Hematologic and Oncology and Respiratory Medicine, University of Muenster, Muenster, Germany.

[2] Institute of Pathology, Bergmannsheil-University Hospital, Bochum, Germany.

[3] Department of Clinical Chemistry, University of Muenster, Muenster, Germany.

[4] Department of Biomathematics, University of Muenster, Muenster, Germany.

[5] Lungenklinik Hemer, Hemer, Germany.

[6] Department of Thoracic Surgery, Krankenhaus St. Raphael, Ostercappeln, Germany.

Address for reprints: Petra Broermann, M.D., Department of Medicine/Hematologic and Oncology and Respiratory Medicine, University of Muenster, Albert-Schweitzer-Strasse 33, 48129 Muenster, Germany; Fax: 49-251/83-47680; E-mail: broerma@uni-muenster.de

In addition to patients from the University Hospital in Muenster, patients were enrolled from three additional institutions of the German Lung Cancer Cooperative Group: Krankenhaus St. Raphael, Osterkappeln (Dr. F. Klinke, Dr. E. Striehn), Lungenklinik Hemer, Hemer (Dr. H.N. Macha, Dr. L. Freitag), and Kreiskrankenhaus Diekholzen, Diekholzen (Dr. D. Vallee, Dr. A. Deimling).

Received July 5, 2001; revision received October 25, 2001; accepted November 9, 2001.

BACKGROUND. In a trimodality treatment approach for Stage III nonsmall cell lung carcinoma (NSCLC), the prognostic impact of the *ras* mutation status in resection specimens was evaluated.

METHODS. Forty patients with Stage III NSCLC underwent tumor resection after neoadjuvant treatment with two cycles of chemotherapy (ifosfamide, carboplatin, and etoposide) and subsequent twice-daily radiotherapy (45 grays [Gy]; 2 × 1.5 Gy/day) with concurrent carboplatin and vindesine. Assessment of K-*ras* codon 12 mutation status was performed in the paraffin embedded resection specimens by a two-step polymerase chain reaction followed by restriction fragment length polymorphism analysis.

RESULTS. K-*ras* mutation status could be assessed in 28 cases. A K-*ras* codon 12 point mutation was found in 13 of 28 resection specimens (46%). The mutation was found independently of gender, age, tumor stage, and clinical response status and occurred more frequently in adenocarcinomas. Even after complete resection, the presence of a K-*ras* mutation was a significant predictor for a poor progression free survival ($P = 0.005$).

CONCLUSIONS. These data suggest that further evaluation of the K-*ras* codon 12 mutation status in trials on neoadjuvant and adjuvant therapy is warranted. This may contribute to the identification of stratification variables for future treatment approaches. ***Cancer* 2002;94:2055–62.** © *2002 American Cancer Society.*
DOI 10.1002/cncr.10387

KEYWORDS: nonsmall cell lung carcinoma, trimodality therapy, prognosis, K-*ras*.

Although in patients with Stage III[1,2] (Stage IIIA: T1-3N2M0; Stage IIB: T4N1-3M0 or T1-4N3M0) nonsmall cell lung carcinoma (NSCLC) and mediastinoscopically proven N2 disease complete tumor resection with radical mediastinal lymph node dissection is feasible, 5-year survival rates do not exceed 10%.[2] This poor outcome is because of competing risks of early systemic and/or local recurrence. The introduction of neoadjuvant treatment, consisting of chemotherapy or chemoradiotherapy, or chemotherapy followed by simultaneous radiochemotherapy seems to enable an improvement of prognosis.[3] Nonetheless, in these studies treatment-related mortality rates ranged from 5% to 15%, when using radiotherapy preoperatively in combination with chemotherapy.[3] Moreover, even with this treatment, systemic recurrence is still a problem. Thus, it is an important issue to identify patient subgroups with the best conditions to benefit from this strenuous kind of therapy, and conversely, to select patients with a high risk of recurrence for a modified and more effective treatment.

Recent advances in molecular biology led to the identification of various molecular genetic features being considered as powerful determinants of prognosis in NSCLC. One of the most frequently documented alterations correlating with poor prognosis is the mutation of the K-*ras* oncogene.[4,5] In the neoadjuvant treatment approach, only one randomized study assessed the K-*ras* mutation status in the resection specimens of Stage IIIA patients receiving neoadjuvant chemotherapy before surgery or surgery alone.[6] The current study investigated the prognostic impact of a K-*ras* codon 12 point mutation in the resection specimens of patients with Stage III NSCLC receiving chemotherapy followed by simultaneous radiochemotherapy and then surgery.

The *Ras* gene family comprises three genes, K-, H- and N-*ras* encoding similar 21-kilodalton membrane-bound proteins $p21^{ras}$. By a point mutation in codons 12 (85% of all K-*ras* mutations), 13, or 61, the oncogene acquires transforming potential. The amino acid change alters the resulting $p21^{ras}$ protein configuration with marked reduction of the intrinsic GTPase activity keeping the protein in an active GTP-bound state. Fixed in this "on" state, RAS proteins switch on a kinase cascade with proteins such as FOS, JUN, or MYC being downstream from the nucleus. Genes activated by these proteins, e.g., cyclin D1, turn on the cell cycle, and cell growth becomes irregular.

In NSCLC, mutations of K-*ras* are found in up to 30% of adenocarcinomas[7] and 8% of squamous cell carcinomas[8] with a higher frequency in advanced stages.[9] Recent studies reported mutation rates up to 50% in adenocarcinomas using an assay detecting 1 mutation among 10^3 alleles.[10] The molecular assessment in the current trial is based on this high sensitive assay and evaluated the prognostic impact of the proof of K-*ras* mutations in resection specimens of Stage III NSCLC patients with preoperative chemotherapy followed by chemoradiation.

PATIENTS AND METHODS

Study Subjects

This study is based on a trimodality treatment approach for Stage III NSCLC. It was approved by the ethics committee of the University of Muenster. Fifty-four patients were recruited from four participating institutions from 1992 to 1995. After giving written informed consent, patients with histologically confirmed NSCLC of Stage IIIA (T1–3,N2,M0) or IIIB (T4,N1–3,M0 or T1–4,N3,M0)[1] were enrolled when fulfilling the following inclusion criteria: a favorable medical condition (Eastern Cooperative Oncology Group [ECOG] performance status of 0 or 1), age between 18 and 69 years, adequate liver and kidney function (serum creatinine $<$ 1.5 mg/dL, bilirubin $<$ 1.5 mg/dL), sufficient bone marrow reserve (leukocytes $>$ 4000/μL and thrombocytes $>$ 100,000/μL), and a predicted postoperative FEV1 greater than 1.0 l. Patients with involvement of supraclavicular lymph nodes or positive pleural effusion were not eligible. Pretreatment evaluation required laboratory investigations, bronchoscopy, chest X-ray, computed tomography (CT) of thorax, abdomen, and brain, and a bone scan, as well as the assessment of the mediastinal lymph nodes, using a mediastinoscopy or an exploratory thoracotomy. Of the 54 enrolled patients, 40 underwent tumor resection (Stage IIIA, $n = 20$; Stage IIIB, $n = 20$). This patient cohort was the subject of further analysis. Of the remaining patients three were judged technically inoperable, two were inoperable for medical reasons, one died preoperatively from pneumonitis, and four patients refused surgery. Moreover, one patient died preoperatively with local tumor progression, and three patients did not receive surgery because of distant metastases after neoadjuvant treatment.

Treatment, Response Assessment, and Follow-Up

Patients underwent a bimodality preoperative therapy consisting of chemotherapy followed by concurrent chemoradiotherapy. First, two cycles of chemotherapy (second cycle started on Day 22) with carboplatin 300 mg/m^2 (Day 1), ifosfamide 1500 mg/m^2 (Days 1, 3, 5), and etoposide 100 mg/m^2 (Days 1, 3, 5) were administered. Beginning on Day 6, patients received subcutaneous injections of granulocyte colony-stimulating factor (300 μg/day) until the leukocyte count exceeded 4000/μL. The following concurrent chemoradiotherapy commenced 3 weeks after the start of the second cycle of chemotherapy with a total irradiation dose of 45 Gy (hyperfractionated and accelerated, e.g., two daily single fractions of 1.5 Gy, 5 days per week). Chemotherapy with carboplatin 100 mg/m^2 and vindesine 3 mg absolute was administered simultaneously on Days 1, 8, and 15. On average, 7 weeks after completing chemoradiotherapy operable patients who remained free of distant metastases underwent radical surgery with curative intent. Patients who were inoperable or had noncurative incomplete tumor resection were further irradiated with conventional fractionation (16 Gy; 5 $\times$ 2 Gy/week).

The preoperative staging was conducted 4 weeks after completion of simultaneous chemoradiotherapy and comprised the initial evaluation without mediastinoscopy. Clinical response was assessed according to the guidelines of the Southwest Oncology Group.[11] Follow-up examinations were taken every 3 months for the first 2 years and all 6 months thereafter.

Histopathologic Assessment

For all 40 patients undergoing surgery, formalin fixed resection samples from the primary lesion and from resected mediastinal lymph nodes were sent to the study's reference pathology center (Institute of Pathology, University Hospital Bergmannsheil, Bochum, Germany) and were examined by the same pathologist. The degree of histopathologic response was determined by assessing the extent of tumor necrosis. As described earlier, a regression grading with four levels of tumor response was derived.[12,13] For further statistical analysis, samples were assigned to the category "tumor regression greater than 90%," when less than 10% of residual tumor cells were present in the paraffin embedded slides of the resection specimens and to the category "tumor regression less than 90%," if greater 10% of residual tumor cells were present.

Molecular Analysis of K-*ras*

The presence of a K-*ras* codon 12 mutation was examined by a combined two-step polymerase chain reaction (PCR) followed by an analysis of the restriction fragment length polymorphism (RFLP). This approach enables detection of 1 mutated allele among 10^3 normal alleles (sensitivity 0.1%).[14] The first step of PCR amplified the K-*ras* codon 12 region; the second step selectively amplified the mutated allele. The first PCR (primers A, B) led to a fragment with two restriction sites for the restriction enzyme Mva1 in case of missing mutations in codon 12 and to one restriction site if codon 12/1 or 12/2 contained a point mutation. The second PCR performed with primers A and C amplified a shortened fragment, containing one restriction site with wild-type codon 12 and failing to cleave if there was a point mutation.[14]

For DNA preparation from the formalin fixed paraffin embedded material, 10-μm sections were cut, deparaffinized with xylol and ethanol, and then dried. Thereafter, the DNA was isolated using proteinase K and a Qiamp DNA kit according to the manufacturer's specifications (Qiagen, Hilden, Germany).

Amplifications with Taq polymerase were performed in 50-μL reaction mixtures containing 1.5 U of polymerase, 25 pmol of each primer (A, 5′-3′: ACTGAATATAAACTTGTGGTAGTTGGACCT; B, 5′-3′: TCAAAGAATGGTCCTGGACC; C, 5′-3′: TAATATGTCGACTAAAACAAGATTTACCTC), dATP, dCTP, dGTP, and dTTP, 1.5 mM Mg2+, 6.0 mM KCl, and 10 mM Tris-HCl (pH 8.8). Overlaid with 25 μL mineral oil, samples were subjected to amplification. Each cycle stepped from 94 °C for 60 seconds to 55 °C for 60 seconds and to 72 °C for 70 seconds. The first-round PCR comprised 30 cycles and the second round 35 cycles, each followed by incubation with Mva1 (Eurogentec, Seraing, Belgium) for a time period of 2 hours at 37 °C. Each assay contained a sample with water as a negative control and pancreatic carcinoma cells with known codon 12 mutation as a positive control.

Then, to verify the validity of the method, in some cases showing a K-*ras* point mutation in PCR/RFLP, the kind of mutation was determined using a fluorescence-based DNA minisequencing method: First a DNA fragment spanning the site of the mutation was amplified using one biotinylated and one unbiotinylated primer. The amplified fragment—then carrying a biotin residue in one of its strands—was captured on a solid support with the aid of the biotin–streptavidin interaction. The unbiotinylated strand then was denatured. In the minisequencing reaction—using the remaining biotinylated immobilized strand—an oligonucleotide primer, complementary to the DNA region immediately adjacent to the site of the mutation was able to hybridize to the immobilized strand. Then a DNA polymerase was used to specifically extend the primer with one single labeled tritium-marked dideoxy nucleoside triphosphate. The complementary nucleotide was incorporated whereas the noncomplementary nucleotides were not. After the reaction, the incorporated label was measured in the scintillation counter.[15]

Statistical Analysis

Statistical analysis was performed using SAS for Windows 6.11 (SAS Institute, Cary, NC). All survival curves were calculated according to Kaplan and Meier.[16] For progression free survival, the difference between the date of the primary diagnosis and progression of disease or death was used. For overall survival, the difference between the date of the primary diagnosis and death was used. Subsequently, curves were analysed using the log-rank test. The four-field table Fisher exact test was used to assess the relation between two variables each with two categories. To estimate the prognostic significance of covariables on progression free survival, we performed an exploratory multivariate analysis by using the stepwise Cox regression model.[17] The significance in all statistical tests was set at P value less than 0.05.

RESULTS

Clinical Response, Resectability, and Histopathologic Response in Resection Specimens of Patients Undergoing Surgery

In the group of 40 surgically treated patients, 28 showed a partial response, 4 showed a complete clinical response, and 8 showed "no change" before surgery[11] (Table 1). Tumor resection was scored complete

TABLE 1
Patient Characteristics, Response to Treatment, Resectability, and Histopathologic Response of Patients Undergoing Tumor Resection (n = 40)

Characteristic	No.	Percentage
Age (yrs)		
Median	57	
Range	37–69	
Gender		
Male	36	90
Female	4	10
Performance status (ECOG)		
0	25	63
1	15	37
Histologic type		
Adenocarcinoma	12	30
Squamous cell carcinoma	28	70
Tumor stage		
IIIA	20	50
IIIB	20	50
Clinical response		
Complete response/partial response	4/28	10/70
No change/progressive disease	8/0	20/0
Resectability		
R0	34	85
R1/R2	4/2	10/5
Histopathologic response		
Pathologic complete response	7	18
Tumor regression > 90%	20	50
Tumor regression < 90%	13	32

ECOG: Eastern Cooperative Oncology Group.

(R0, resection margin tumor free) in 34 patients (Stage IIIA, n = 17; Stage IIIB, n = 17) and incomplete in 6 patients. Twenty-seven of the 40 resection samples (Stage IIIA, n = 14; Stage IIIB, n = 13) showed a marked histopathologic response with less than 10% residual tumor tissue (tumor regression > 90%). Seven of these samples revealed a histopathologic complete response. Thirteen specimens showed a tumor regression of less than 90%; 3 of these without any therapy-induced histologic tumor response (Table 1). There was no statistically significant correlation between the tumor regression (< 90% vs. > 90%) and histology (adenocarcinoma vs. squamous cell carcinoma), age (younger than 60 years vs. older than 60 years), gender, stage (IIIA vs. IIIB), or the preoperative clinical response status (complete response/partial response vs. no change/progressive disease; Fisher exact test).

Molecular Analysis

From the total of 40 resection specimens in 28 cases, a molecular assessment could be performed. Seven samples—with histopathologic complete response—contained no tumor tissue for further assessment. Nevertheless, to confirm histopathologic complete response by means of molecular analysis we performed in an exploratory approach K-*ras* assessment in three of these seven samples, without detection of a K-*ras* mutation. In five cases with subtotal therapy-induced tumor regression, it was not possible to prepare histologic slides bearing sufficient tumor tissue for further molecular biologic investigations. A K-*ras* codon 12 point mutation was detected in 13 of 28 cases (46%). In 5 of the 13 positive specimens, a PCR minisequencing was performed for validation of the K-*ras* mutation as determined by PCR/RFLP. In all five cases, the K-*ras* mutations were confirmed and specified: three were G → T-point mutations, two in codon 12/1 and one in codon 12/2, and the remaining two were G → C-mutations in codon 12/1.

The K-*ras* mutations, detected by PCR/RFLP, were found independently of gender, age, tumor stage, and clinical response status (Fisher exact test). The mutation occurs more frequently in adenocarcinomas (6 of 8) compared with squamous cell carcinomas (7 of 20) and in cases with a minor extent of histopathologic response (tumor regression < 90%; 8 of 13) compared with cases with a tumor regression greater 90% (5 of 15), but this difference is without statistical significance. In patients with incomplete tumor resection (R1/R2 resection), the K-*ras* mutation was detected significantly more often than in patients with R0 resection (4 of 4 vs. 9 of 24) (P = 0.035, Fisher exact test; Table 2).

Survival Analysis

With a median follow-up period of 44 months, the median survival time for all patients was 20 months (3-year survival rate, 30%) and 23 months for patients undergoing resection (n = 40; 3-year survival rate, 36%). In univariate analyses neither age (younger than 60 years vs. older than 60 years), histology (adenocarcinoma vs. squamous cell carcinoma), performance status (ECOG 0 vs. ECOG 1), nor stage (IIIA vs. IIIB) revealed as significant predictors for survival. However, tumor regression in resection specimens (> 90% vs. < 90%) was a significant predictor for survival (P = 0.02). Even in patients with complete resection, this could be shown (median survival, not reached vs. 23 months; 3-year survival rate, 56% vs. 11%; P = 0.03, log-rank test).

The presence of a K-*ras* codon 12 point mutation in the resected tumor tissue (K-*ras* mutation vs. K-*ras* wild type) became apparent as a predictor for a shortened progression free survival (median, 9 vs. 21 months; 3-year survival rate, 0% vs. 40%; P = 0.003). Yet, for overall survival, this remained without statistical significance (P = 0.07). In patients with complete

TABLE 2
Assessment of K-*ras* Mutation Status in Resection Specimens According to Histology, Tumor Stage, Response to Treatment, Resectability, and Histopathologic Response

	K-*ras* mutation status (n = 28)	
Characteristic	Positive[a] (n = 13)	Negative[b] (n = 15)
Histologic type		
Adenocarcinoma	6	2
Squamous cell carcinoma	7	13
Tumor stage		
IIIA	8	7
IIIB	5	8
Clinical response		
CR/PR	10	12
NC/PD	3	3
Resectability		
R0	9	15
Non-R0	4	0
Histopathologic response		
Tumor regression > 90%	5	10
Tumor regression < 90%	8	5

R0: complete tumor resection with microscopic tumor free resection margins; non-R0: tumor resection microscopically incomplete or with macroscopic residual disease; CR: complete response; PR: partial response; NC: no change; PD: progressive disease.
[a] K-*ras* codon 12 point mutation assessed by polymerase chain reaction/restriction fragment length polymorphism.
[b] Wild type.

resection, the impact of the K-*ras* mutation status on progression free survival could be shown as well (median, 10 vs. 21 months; 3-year survival rate, 0% vs. 40%; $P = 0.005$; Table 3). Pursuing further analysis in patients with completely resected tumors, grouped according to the extent of histopathologic response, revealed the following: in patients with tumor regression < 90% the K-*ras* mutation status became statistically significant for progression free survival (median, 10 vs. 21 months; 3-year survival rate, 0% vs. 20%; $P = 0.03$). In patients with tumor regression greater than 90% the P value could not be determined because of inhomogeneous distribution of K-*ras* mutation status (log-rank test). Nonetheless, all 3 K-*ras* mutated patients in this group had tumor progression within 16 months, whereas the median of progression free survival was not reached in the nonmutated patients ($n = 10$).

DISCUSSION

In a trimodality treatment approach, the current study investigated the prognostic impact of the detection of a K-*ras* mutation in resection specimens of patients with Stage III NSCLC. In the recent past, several trials using induction with preoperative chemoradiation in Stage III NSCLC reported complete resection,[18] a favorable histopathologic response in mediastinal lymph nodes,[19,20] or in mediastinal lymph nodes and resection specimens of the primary tumor[13] as being significant predictors of survival. As already reported,[13] in the current study for patients with complete resection the extent of histopathologic response (tumor regression > 90% vs. tumor regression < 90%) had a significant impact on survival (median, not reached vs. 23 months; 3-year rate, 56% vs. 11%; $P = 0.03$). In this report, in addition it is demonstrated that in this treatment setting for patients with complete resection the demonstration of a K-*ras* codon 12 point mutation in the resected tumor tissue is predictive of an unfavorable progression free survival time (median, 10 vs. 21 months; $P = 0.005$). Moreover, it turned out that in patients with completely resected tumors with an unfavorable histopathologic response, the K-*ras* mutation status significantly discriminates in terms of progression free survival (median, 10 vs. 21 months; $P = 0.03$). Thus, particularly in this patient group, the K-*ras* mutation status has additional prognostic impact beyond histopathologic response in this treatment setting with bimodality induction. Even in the patient subgroup with complete resection and a favorable histopathologic response, it became suggestive that the demonstration of a K-*ras* codon 12 point mutation has an adverse impact on progression free survival.

However, unavailability of molecular K-*ras* assessment of those 14 patients not undergoing surgery potentially could have confounding effects on the results and the prognostic impact demonstrated. Particularly, if those 14 patients or a substantial portion of these patients would have been omitted from surgery because of disease progression, then this group would represent the most adverse prognostic subset and additionally would have been excluded from further analysis; thus, potentially affects with a bias on the results. Nonetheless, because only four patients did not undergo surgery because of progression disease, we would not consider such a bias, tackling the prognostic impact demonstrated.

Among the molecular markers assessed in terms of prognosis in NSCLC, the significance of a K-*ras* mutation was documented extensively. In several studies K-*ras* mutation was associated with significantly reduced overall or progression free survival.[5,7,21–23] However, these studies generally were retrospective, included small patient groups,[7,9] were heterogeneous in terms of tumor classification,[5,7,21,22] or used NSCLC cell lines obtained over a long time period.[23] Furthermore, the varying sensitivity of PCR techniques used led to a reduced comparability of the

TABLE 3
Progression Free Survival According to K-*ras* Mutation Status (K-*ras* Positive vs. K-*ras* Negative) in Patients with Complete Resection and Subdivided in Favorable Histopathologic Response (Tumor Regression > 90%) and Unfavorable Histopathologic Response (Tumor Regression < 90%)

	Progression free survival				
	Median (mos)		3-year rate (%)		
Patient type	K-*ras* pos.	K-*ras* neg.	K-*ras* pos.	K-*ras* neg.	*P* value[a]
R0-resected tumors (n = 24)	10	21	0	40	0.005
R0-resected tumors with tumor regression > 90% (n = 13)	12	Not reached	0	50	—[b]
R0-resected tumors with tumor regression < 90% (n = 11)	10	21	0	20	0.03

[a] Determined with median survival.
[b] Because of inhomogeneous distribution of K-*ras* mutation status, *P* value could not be determined.

findings.[10] Moreover, in a further study, K-*ras* mutation was not predictive of survival in Stages I and II of NSCLC, but in a subgroup analysis of Stage III.[9] Recently, in a prospective trial of 184 patients with primary resected tumors with Stage II and IIIA NSCLC undergoing postoperative radiotherapy or chemoradiation the prognostic impact of K-*ras* mutation was weak with marginally statistical significance only in multivariate analysis ($P = 0.066$).[24] Therefore, these analyses on the prognostic impact of K-*ras* mutation are still controversial and all have been performed in the resection specimens of previously untreated patients. One further trial,[6] comparing surgery with or without preoperative chemotherapy (mitomycin, ifosfamide, and cisplatin), assessed the K-*ras* mutation status in resection specimens after preoperative induction. The preoperatively treated patients had a significant better overall and progression free survival rate ($P < 0.001$ each). A K-*ras* mutation was demonstrated significantly more often in the group receiving surgery alone (10 of 24 vs. 3 of 20; $P = 0.05$).[6] Possibly, an inhomogeneous distribution of K-*ras* positive patients contributed to these different survival rates. Conversely, the lower K-*ras* mutation rate in the chemotherapy group could be a result of effective preoperative induction, eliminating tumor compartments with mutated K-*ras*. Nonetheless, experimental evidence did not support this assumption, because the transfection of mutated RAS genes into NIH-3T3 mouse fibroblasts led to less sensitivity to radiation and to resistance against different cytotoxic agents, e.g., cisplatin.[25,26] It has been suggested that the promoter of *mdr*1 gene, encoding for the p180-transporter protein that is associated with multidrug resistance, is sensitive to activated $p21^{ras}$. However, in human cells a consistent effect could not be induced. Several NSCLC cell lines were tested for resistance against different cytotoxic agents without any association between RAS mutation and resistance.[27] In a prospective trial, 69 patients with inoperable advanced adenocarcinoma of the lung received chemotherapy consisting of ifosfamide, carboplatin, and etoposide. Here, according to K-*ras* mutation status, no difference became obvious in terms of response to chemotherapy or survival rates.[28]

Yet, the impact of RAS mutation–induced radiation resistance remains uncertain in vivo. In the current study, K-r*as* mutations occurred significantly more often in patients with incomplete tumor resection and in patients with an unfavorable histopathologic response. As reported elsewhere, radiochemotherapy had a substantial impact on tumor downstaging, increasing the response rate from 41% after chemotherapy to 69% after radiochemotherapy.[13] Thus, we assume that the radiochemotherapy block is essential for achieving a favorable histopathologic response and currently are testing this hypothesis with a Phase III trial.[29] The finding that K-*ras* mutations occurred more frequently in resection specimens with an unfavorable histopathologic response supports the experimental evidence of radiation resistance in association with a positive K-*ras* mutation status.

In the current study, the proof of a K-*ras* mutation in the resection specimens was predictive of shorter progression free survival. Given the small study population, the analyzed results must be interpreted cautiously. One can speculate that, in a trimodality treatment setting with a larger patient population and with the analysis of K-*ras* mutation status available, the

same predictive effect will become apparent with significant impact on overall survival. Indeed, in patients with resectable NSCLC, some reports point to K-*ras* mutation as being associated with significantly shorter survival.[5,7,21] All these analyses, however, have been performed in the resection specimens of previously untreated patients.

These data and the results of the current analysis suggest that further evaluation of the K-*ras* codon 12 mutation status in trials on neoadjuvant and adjuvant therapy is warranted. This may contribute to the identification of stratification variables for future treatment approaches and selection of patients for a different systemic therapy. Currently, we have no evidence that adjuvant chemotherapy after complete resection of NSCLC has an impact on survival. This has been delineated from a meta-analysis with more than 1000 patients.[30] Thus, we have to be aware that for the proof of any adjuvant intervention in NSCLC we presumably need survival data from thousands of patients. However, for patients after complete resection of tumors with K-*ras* mutations targeted therapy strategies blocking the K-*ras* signaling pathway could evolve as future treatment options. Further activation of the mutated cytosolic RAS protein requires protein modification through farnesylation with translocation to the membrane. Recently, it has been shown that farnesyl transferase inhibitors inhibit the growth of lung carcinoma cell lines by blocking RAS activation and are showing clinical activity in Phase I trials.[31,32] Possibly, the addition of targeted therapy with farnesyl transferase inhibitors may be a perspective to improve the outcome of patients with unfavorable molecular features like *ras* mutations in resection specimens after induction therapy. Even though a straightforward inclusion of these noteworthy drugs in such treatment settings seems to be difficult and large-scale randomized trials are required, testing of this approach may be worthwhile.

REFERENCES

1. Mountain CF. Revisions in the international system for staging lung cancer. *Chest.* 1997;111:1710–1717.
2. Andre F, Grunenwald D, Pignon JP, et al. Survival of patients with resected N2 non-small-cell lung cancer: evidence for a subclassification and implications. *J Clin Oncol.* 2000;18: 2981–2989.
3. Johnson DH, Turrisi AT, Pass HI. Combined modality treatment for locally advanced non-small cell lung cancer. In: Pass HI, Mitchell JB, Johnson DH, Turrisi AT, editors. Lung cancer: principles and practice. 2nd ed. New York: Lippincott; 2000: 910–920.
4. Barbacid M. Ras genes. *Annu Rev Biochem.* 1987;56:799–827.
5. Slebos RJC, Kibbelaar RE, Dalesio O, et al. K-*ras* oncogene activation as a prognostic marker in adenocarcinoma of the lung. *N Engl J Med.* 1990;323:561–565.
6. Rosell R, Gomez-Codina J, Camps C, et al. A randomized trial comparing preoperative chemotherapy plus surgery with surgery alone in patients with non-small-cell lung cancer. *N Engl J Med.* 1994;330:153–158.
7. Rodenhuis S, van de Wetering ML, Mooi WJ, et al. Mutational activation of the K-*ras* oncogene. *N Engl J Med.* 1987; 317:929–935.
8. Vachtenheim J, Horáková H, Opálka P, Roubková H. Mutations of K-*ras* oncogene and absence of H-*ras* mutations in squamous cell carcinomas of the lung. *Clin Cancer Res.* 1995;1:359–365.
9. Cho JY, Kim JH, Lee YH, et al. Correlation between K-*ras* gene mutation and prognosis of patients with non-small cell lung carcinoma. *Cancer.* 1997;79:462–467.
10. Mills NE, Fishman CL, Rom WN, Dubin N, Jacobson DR. Increased prevalence of k-*ras* oncogene mutations in lung adenocarcinoma. *Cancer Res.* 1995;55:1444–1447.
11. Green S, Weiss GR. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. *Invest New Drugs.* 1992;10:243–253.
12. Junker K, Thomas M, Schulmann K, Klinke F, Bosse U, Müller KM. Tumour regression in non-small-cell lung cancer following neoadjuvant therapy. Histological assessment. *J Cancer Res Clin Oncol.* 1997;123:469–477.
13. Thomas M, Rübe C, Semik M, et al. Impact of preoperative bimodality induction including twice-daily radiation on tumor regression and survival in stage III non-small-cell lung cancer. *J Clin Oncol.* 1999;17:1185–1193.
14. Levi S, Urbano-Ispizua A, Gill R, et al. Multiple K-*ras* codon 12 mutations in cholangiocarcinomas demonstrated with a sensitive polymerase chain reaction technique. *Cancer Res.* 1991;51:3497–3502.
15. Syvänen AC. Detection of point mutations in human genes by the solid-phase minisequencing method. *Clin Chim Acta.* 1994;226:225–236.
16. Kaplan EL, Meier P. Non-parametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457–481.
17. Cox DR. Regression models and life tables [with discussion]. *J Royal Stat Soc.* 1972;B34:187–220.
18. Eberhardt W, Wilke H, Stamatis G, et al. Preoperative chemotherapy followed by concurrent chemoradiation therapy based on hyperfractionated accelerated radiotherapy and definitive surgery in locally advanced non-small cell lung cancer: mature results of a phase II trial. *J Clin Oncol.* 1998;16:622–634.
19. Albain KS, Rusch VW, Crowley JJ, et al. Concurrent cisplatin/etoposide plus chest radiotherapy followed by surgery for stages IIIA (N2) and IIIB non-small-cell lung cancer: mature results of Southwest Oncology Group phase II study 8805. *J Clin Oncol.* 1995;13:1880–1892.
20. Choi NC, Carey RW, Daly W, et al. Potential impact on survival of improved tumor downstaging and resection rate by preoperative twice-daily radiation and concurrent chemotherapy in stage IIIA non-small-cell lung cancer. *J Clin Oncol.* 1997;15:712–722.
21. Rosell R, Li S, Skacel Z, et al. Prognostic impact of mutated K-*ras* gene in surgically resected non-small cell lung cancer patients. *Oncogene.* 1993;8:2407–2412.

22. Fukuyama Y, Mitsudomi T, Sugio K, Ishida T, Akazawa K, Sugimachi K. K-*ras* and *p53* mutations are an independent unfavorable prognostic indicator in patients with non-small-cell lung cancer. *Br J Cancer.* 1997;75:1125–1130.
23. Mitsudomi T, Steinberg SM, Oie HK, Mulshine JL, Phelps R, Viallet J. *ras* gene mutations in non-small cell lung cancers are associated with shortened survival irrespective of treatment intent. *Cancer Res.* 1991;51:4999–5002.
24. Schiller JH, Adak S, Feins RH, Keller SM, Fry WA, Livingston RB. Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small cell lung cancer on E4592: a laboratory ancillary study on an Eastern Cooperative Oncology group prospective randomized trial of postoperative adjuvant therapy. *J Clin Oncol.* 2001;19:448–457.
25. Sklar MD. The ras oncogenes increase the intrinsic resistance of NIH 3T3 cells to ionizing radiation. *Science.* 1988; 239:645–647.
26. Sklar MD. Increases resistance to cis-diammine-dichlorpatinum(II) in NIH 3T3 cells transformed by *ras* oncogenes. *Cancer Res.* 1988;48:793–797.
27. Tsai CM, Chang KT, Perng RP, et al. Correlation of intrinsic chemoresistance of non-small cell lung cancer cell lines with HER-2/neu gene expression but not with RAS gene mutations. *J Natl Cancer Inst.* 1993;85:897–901.
28. Rodenhuis S, Boerrigter L, Top B, et al. Mutational activation of the K-*ras* oncogene and the effect of chemotherapy in advanced adenocarcinoma of the lung: a prospective study. *J Clin Oncol.* 1997;15:285–291.
29. Thomas M, Rübe C, Semik M, et al. Randomized trial of chemotherapy and twice-daily chemoradiation versus chemotherapy alone before surgery in stage III non-small cell lung cancer. Interim analysis of toxicity [abstract]. *Proc Am Soc Clin Oncol.* 1999;18:458a.
30. Non-Small Cell Lung Cancer Collaborative Group. Chemotherapy in non-small cell lung cancer: a meta-analysis using updated data on individual patients from 52 randomised clinical trials. *Br Med J.* 1995;311:899–909.
31. Adjei AA, Erlichman C, Davis JN, et al. A phase I trial of the farnesyl transferase inhibitor SCH66336: evidence for biological and clinical activity. *Cancer Res.* 2000;60:1871–1877.
32. Zujewski J, Horak ID, Bol CJ, et al. Phase I and pharmacokinetic study of farnesyl protein transferase inhibitor R115777 in advanced cancer. *J Clin Oncol.* 2000;18:927–941.